In re Application of: Wahi and O'Gorman

Application No.:

10/086,542

Filing Date:

February 28, 2002

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PATENT Attorney Docket No.: SALK1790-6

(088802-3457)

The FLP recombination target site (sometimes referred to herein as "FRT") has also [0028]been identified as minimally comprising two 13 base-pair repeats, separated by an 8 base-pair spacer, as follows:

-Spacer-5' - GAAGTTCCTATTC[TCTAGAAA]GTATAGGAACTTC - 3' (SEQ ID NO:3) Xba I site

The nucleotides in the above "spacer" region can be replaced with any other combination of nucleotides, so long as the two 13 base-pair repeats are separated by 8 nucleotides. The actual nucleotide sequence of the spacer is not critical, although those of skill in the art recognize that, for some applications, it is desirable for the spacer to be asymmetric, while for other applications, a symmetrical spacer can be employed. Generally, the spacers of the FLP recombination target sites undergoing recombination with one another will be the same.

Please replace paragraph [0047] on page 14 with the following replacement paragraph:

pFRT β GAL contains a version of the bacterial β -galactosidase sequence modified by [0047] insertion of a FLP recombination target site, or FRT, within the protein coding sequence immediately 3' to the translational start. The oligonucleotide used for the construction of pFRTβGAL was:

5' - GATCCCGGGCTACCATGGA GAAGTTCCTATTC CGAAGTTCCTATTC (TCTAGA)AAGTATAGGAACTTCA - 3' (SEQ ID NO:4).

This oligonucleotide contains an in-frame start codon, minimal FRT site, and an additional copy of the 13-bp FRT repeat [°XXX°]; the XbaI site within the FRT spacer is enclosed in parentheses. The linker was inserted between the BamHI and HindIII sites of pSKS105 (Casadaban et al., Meth. Enzymol. 100:293, 1983) and the LacZ portion of modified gene was cloned into a pSV2 vector. The neomycin cassette used for construction of pNEOβGAL was an XhoI to BamHI fragment from pMC1neo-polyA (Thomas and Capecchi, Cell 51:503, 1987) cloned between copies of the J3 FRT site in pUC19.



023.218976.1